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A MICROAUTORADIOGRAPHIC STUDY OF THE TRANSLOCATION OF ^{14}C-LABELLED ASSIMILATES IN *ERAGROSTIS CURVULA* (SCHRAD.) NEES

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Abstract

Microautoradiography was used to determine the cellular distribution of translocated ^{14}C-labelled assimilates in *Eragrostis curvula*. The assimilates were labelled by allowing a single leaf to photosynthesize in an atmosphere containing radioactive carbon-dioxide. It was found that the phloem was the main tissue involved in translocation; there was radial translocation from the phloem to other tissues; and some ^{14}C was transported, apparently in the gaseous form, through the lysigenous cavities of vascular bundles.

Uittreksel

'N MIKRO-OUTORADIOGRAFIESE STUDIE VAN DIE TRANSLOKASIE VAN ^{14}C-GEMERKTE ASSIMILATE IN *ERAGROSTIS CURVULA* (SCHRAD.) NEES

Mikro-outoradiografie is gebruik om die sellulêre verspreiding van getranslokeerde ^{14}C-gemerkte assimilate in *Eragrostis curvula* te bepaal. Die assimilate is gemerk deur 'n enkele blaar toe te laat om te fotosintetiseer in 'n atmosfeer wat radioaktiewe koolstofdioksied ($^{14}CO_2$) bevat. Dit is bevind dat die translokasie hoofsaaklik in die floëem plaasgevind het; daar was radiale translokasie van die floëem na die ander weefsels; van die ^{14}C is blykbaar in 'n gastoestand deur die lisigene holtes van die vaatbondels vervoer.

Introduction

The distribution patterns of translocated ^{14}C-labelled photosynthate in grasses have been investigated mainly by gross or "whole plant" autoradiographic methods, eg. Williams, 1964; Sagar and Marshall, 1966; Marshall, 1967; Barnabas and Steinke, 1975; and others. There is little information, however, about the distribution of translocated photosynthate within the tissues of these plants. The aim of the present study was to investigate the distribution of translocated ^{14}C-labelled photosynthate at the cellular level in *Eragrostis curvula* by microautoradiographic techniques. Since a large proportion of photosynthate is water soluble, techniques to prevent leaching of these soluble compounds prior to and during microautoradiography were used.

Material and Methods

Seeds of *Eragrostis curvula* (cultivar Ermelo) were germinated in moist sand. When the seedlings reached the two-tiller stage they were transferred to 1 ℓ bottles

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covered with aluminium foil. The bottles contained a modified Hoagland nutrient solution. The plants remained in a glasshouse under conditions similar to those reported earlier (Barnabas and Steinke, 1975). Plants at the early flowering stage (before anthesis) were used in this study.

The method of introducing ^{14}C into the plants was similar to that described previously (Barnabas and Steinke, 1975). One hundred microcuries of $^{14}CO_2$, generated from $Na_2{}^{14}CO_3$ (specific activity 55 mc/mm), were offered to the third leaf on the culm for 20 min. After a further 30 min photosynthesis in unlabelled CO_2, the plants were harvested.

Samples for microautoradiography were taken from four different parts of the plant (see Fig. 1), namely, from the lower part of the treated leaf (TL) which was not enclosed in the assimilation chamber; from the internode below the node of insertion of the treated leaf; and from two parts of the internode above the node of insertion of the treated leaf. Samples, not exceeding 2 mm in thickness, were taken from each part and were frozen immediately in a mixture of isopentane and dry ice.

A simple, high vacuum freeze-drying apparatus similar to that described by Jensen (1962), was used to freeze-dry the samples. During freeze-drying, the samples were kept frozen by immersing the dehydration chamber in a mixture of dry ice and 65 % ethanol.

Freeze-drying was allowed to continue until the material appeared whitish in colour (ca. 96 h). According to Jensen (1962) the whitish colour of the samples is an indication that the dehydration process is complete. The samples were then infiltrated with paraffin wax under vacuum according to the procedure given by Glick and Malstrom (1952). However, during paraffin wax infiltration only about one third of the samples sank. Those that did not sink were left in molten wax in an oven. After a week about half of these sank. Those that did not sink were discarded. Samples were embedded in fresh filtered paraffin wax according to standard embedding procedures and transverse sections (10 μm thick) were cut on a sledge microtome.

Autoradiographic plates were prepared by wet-mounting pieces of Kodak AR.10 stripping film, with the emulsion side upward on subbed glass slides, i.e. slides coated with a thin layer of gelatin. Mounting of sections on to autoradiographic plates and processing of the plates after exposure were based on methods modified from Haasbroek, Noggle and Fleming (1962). The autoradiographic plates were developed in Kodak D–19b developer and fixed in Kodak acid fixer. Well-preserved sections, superimposed upon their developed images were mounted in a medium having a refractive index of 1,525. Coverslips were applied and the microautoradiographs examined by bright-field microscopy and photographed.

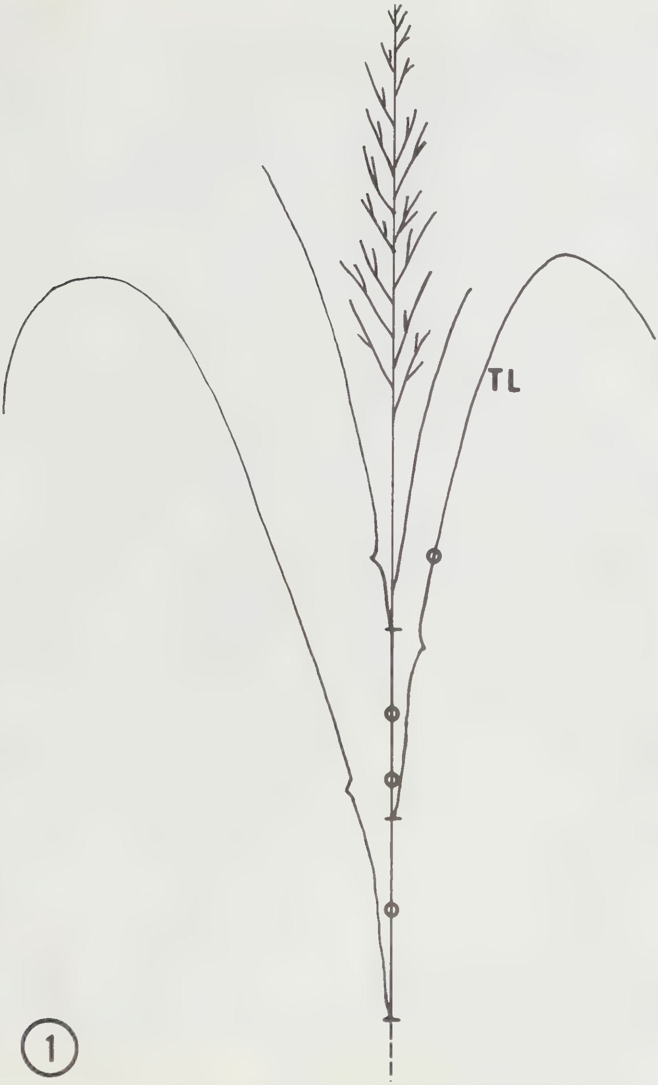


FIG. 1
Diagrammatic representation of part of a plant of *Eragrostis curvula*. Circles indicate portions from which samples were taken for microautoradiography. TL, treated leaf.

RESULTS

The distribution of ^{14}C-labelled assimilates within the tissues of samples taken from four different parts of the plant is shown in Figs 2–5. These microautoradiographs are representative of many microautoradiographs obtained.

Figure 2 shows the distribution of radioactivity in a portion of a vascular bundle of the treated leaf. Although there was too much radioactivity in this section for good resolution, it can clearly be seen that ^{14}C is localized in the phloem. Radioactivity is heavily concentrated in two phloem elements, with smaller amounts in other phloem elements as well.

Figure 3 reveals the presence of radioactivity in parenchyma cells of a section of the internode below the node of insertion of the treated leaf. The resolution of this microautoradiograph is better. Although radioactivity is fairly evenly distributed, a clumping of silver grains is seen in parts of many cells. Labelled assimilates were also detected in the phloem of a vascular bundle near these parenchyma cells.

In Figure 4 the distribution of radioactivity in a vascular bundle and the surrounding tissues of a section of the internode above the node of insertion of the treated leaf, is shown.

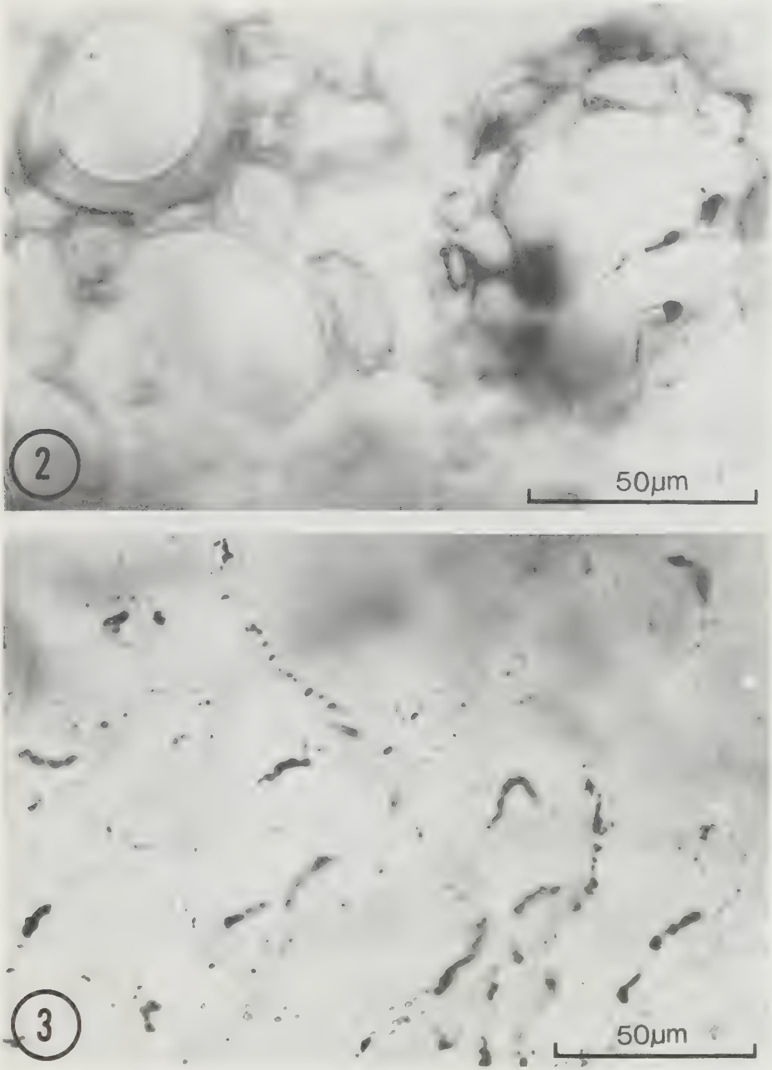
Figure 5 shows ^{14}C concentrated mainly around the lysigenous cavity area of the xylem. This section was taken from the middle of the internode above the treated leaf.

DISCUSSION

Since no radioactivity was present in the xylem of the treated leaf, ^{14}C-labelled photosynthate was translocated from the treated leaf through the phloem. In a detailed investigation of the nature of the substances translocated in *E. curvula* (unpublished work) sucrose was found to be the principal compound exported from the treated leaf. Therefore the radioactivity in the phloem elements was probably due to ^{14}C-sucrose. It is interesting to note that not all phloem elements were involved in translocation during the time of the experiment. This finding is in agreement with that obtained by Trip and Gorham (1967) in squash. In contrast Geiger, Saunders and Cataldo (1969) observed that nearly all sieve tube elements in sugar beet petioles translocated ^{14}C-labelled assimilates.

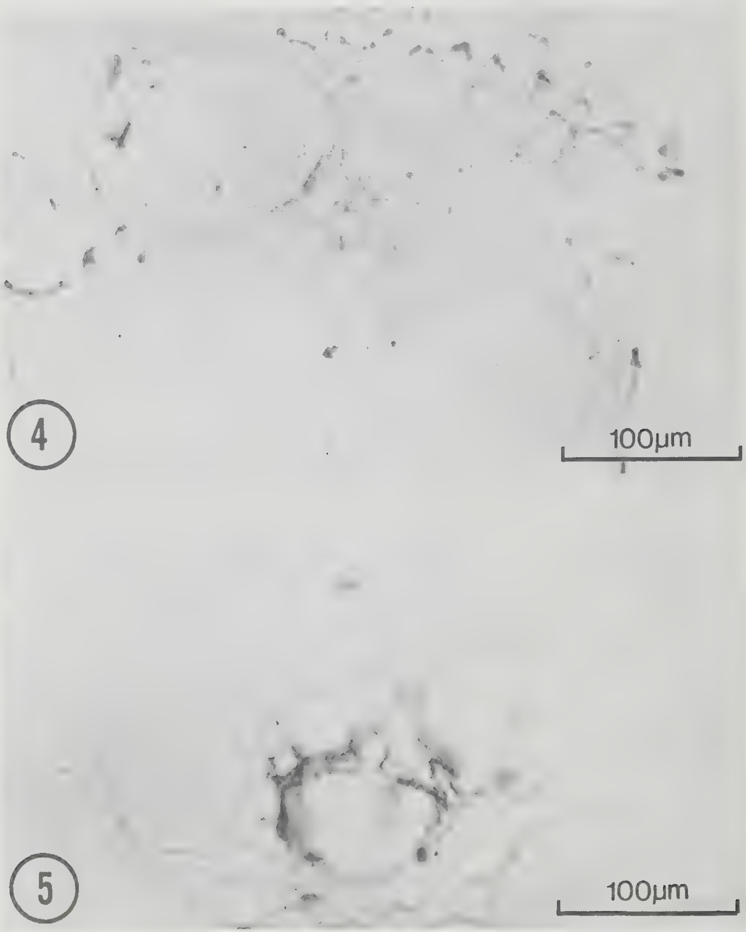
The presence of radioactive material in parenchyma cells of the internode below the treated leaf showed that radial translocation had probably occurred. Radial translocation was probably also responsible for the presence of labelled material around the tissues of a vascular bundle from a section of the internode above the treated leaf. Such movement could have occurred, since a period of 50 min elapsed from exposure to $^{14}CO_2$ till harvest.

In both these microautoradiographs (Figs 3 and 4) a clumping of silver grains was observed in parts of many cells. Trip and Gorham (1967) also noted clumps of silver grains in radial sections showing the distribution of radioactivity in sieve



Figs 2–3

Microautoradiographs of transverse sections. 2. The treated leaf showing ^{14}C localized in the phloem of a vascular bundle. 3. ^{14}C is localized in parenchyma cells of the internode below the treated leaf.



FIGS 4–5

Microautoradiographs of transverse sections. 4. The internode above the treated leaf showing the distribution of ^{14}C within and around a vascular bundle. 5. ^{14}C is localized around the lysigenous cavity of a vascular bundle in the middle of the internode above the treated leaf.

tube elements of squash. The authors suggested that this clumping may be due to the irregular displacement of the cytoplasm during the freezing and embedding procedures. Such a phenomenon may have occurred in this study as well.

The localization of radioactivity mainly around the lysigenous cavity in sections taken from the middle of the internode above the treated leaf could be an artifact of preparation, but is more probably due to the gaseous movement of $^{14}CO_2$ through the lysigenous cavity. Lysigenous cavities, found in many members of the Gramineae, are defined by Metcalf (1960), as longitudinal air canals formed by the breakdown of protoxylem vessels. It is possible that some $^{14}CO_2$, while in transit through a lysigenous cavity, could have gone into solution on contact with the wet walls of the cells surrounding this cavity.

Long distance gaseous movement through intercellular air spaces has been demonstrated by Barber, Ebert and Evans (1962) and by Yoshida and Broadbent (1975). The former investigators showed that air labelled with ^{15}O moved from the shoot to the root in rice and barley plants by gaseous diffusion through continuous intercellular air spaces. Yashida and Broadbent (1975) showed that ^{15}N-labelled nitrogen gas also moved from the shoot to the root in rice plants. The movement of $^{14}CO_2$ in intercellular air spaces was demonstrated by Penny and Nelson (1970) in leaves of soyabean and *Pelargonium*. These investigators showed by whole leaf autoradiography that ^{14}C, applied to localized areas of the leaves, first moved through the intercellular air spaces of the mesophyll as $^{14}CO_2$ before being fixed in photosynthesis. These authors also suggested that the generation of $^{14}CO_2$ in the assimilation chamber around the treated leaf, would favour gaseous movement through the air spaces since there would be a slight increase in pressure and a concentration gradient would also be established. The latter conditions existed in the present investigation when the treated leaf assimilated $^{14}CO_2$.

This study has shown that the movement of $^{14}CO_2$ through air space systems, during translocation experiments, may be possible and further investigation in this direction is needed.

ACKNOWLEDGEMENT

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